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Response of the Lung after Pneumonectomy in Alloxan Diabetic Rats

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ABSTRACT

In order to determine the response of remaining lung after pneumonectomy during diabetes, female Wistar rats that received an intraperitoneal injection of alloxan (D Group) or saline (C Group) were submitted to lung resection. Six days after alloxan injection (45 mg/kg), diabetes was confirmed. The rats were randomized divided in 3 subgroups: non-operated (NO), sham operation (SO) and left pneumonectomy (PE). Thirty days after the surgery all rats were killed and the weight and volume of the lungs were measured. PE-diabetic rats showed smaller lung weight and volume than PE-non-diabetic animals. The results demonstrated that the compensatory lung growth postpneumonectomy were not observed in diabetic rats.

Key Words: Experimental diabetes, Alloxan, Pneumonectomy, Lung growth, Rats, Surgery

INTRODUCTION

Surgery, including lung resection, suppresses insulin secretion and stimulates the release of counter-regulatory hormones resulting in a strong catabolic flux. Normally, these changes are of little clinical significance, but in diabetic patients, catabolism is enhanced by absolute or relative insulin deficiency (Hansen et al., 1989; Sandler et al., 1987). These metabolic changes are not favorable to the compensatory lung growth after pneumonectomy. On the other hand, *diabetes mellitus* (*DM*) is commonly associated with unusual blood levels of growth hormone (GH) and adrenal steroids, hormones known to modulate compensatory lung growth postpneumonectomy (Cahill, 1977). There are only few studies about compensatory lung growth after pneumonectomy during *diabetes*. Ofulue and Thurlbeck (1995) investigated lung growth 7 days after pneumonectomy in streptozotocin-diabetic rats. However, this short period of investigation was not enough to obtain clear results. Therefore, in this work we decided to expand this period of time by assessing the process of compensatory growth in the remaining lung of diabetic rats, i.e., 30 days after pneumonectomy.

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MATERIALS AND METHODS

Ninety female Wistar rats, weighing 210±20g were used. They were kept in a room with constant temperature (23±2ºC). Water and food were given *ad libitum*. The studies were performed with permission of the UNIFESP animal welfare committee.

To induce *DM*, rats were starved for 24 h and alloxan monohydrate (45 mg/kg, dissolved in saline solution at concentration of 45 mg/ml) was injected i.v as described previously (Akimoto et al., 2000). Age-matched rats injected with vehicle alone were employed as control group (C group). Six days after alloxan administration, a sample of blood was collected and glycemia was measured (Bergmeyer and Bernt, 1974). All diabetic animals (D group) with glycemia greater than 200 mg/dL were included in the experiments. C (n=45) and D (n=45) groups were distributed into three subgroups: non-operated (NO/n=15), sham operated (SO/n=15) and left pneumonectomy (PE/n=15). The sham surgery was the control of the anesthetic and surgical trauma. On day 36 all rats were anesthetized with ketamine (i.m) and placed into a plastic box with an oxygen overflow (1 L/min) during 10 min. The surgery was performed under spontaneous ventilation, without oro-tracheal intubation and with oxygen flow (1 L/min) through a bell glass wrapping up the head of the rat. Nattie et al. (1974) and Rannels et al. (1979) also did not use oro-tracheal intubation, but they used oxygen whenever assisted ventilation was necessary.

The rat was positioned in right lateral decubitus. The antisepsis of the left hemi thorax was performed in an aseptic environment with polyvinylpyrrolidone iodine. A left thoracotomy was executed in the fourth intercostal space by an incision in the skin, subcutaneous tissues, serratus muscle, intercostal muscles and parietal pleura. The SO subgroups (C-SO and D-SO) were submitted to thoracotomy without lung resection and PE subgroups (C-PE and D-PE) were submitted to left pneumonectomy. The recovery of the intra-thoracic sub-atmospheric pressure was obtained by aspiration of the pleural cavity with a catheter as described by Seidel et al. (1997_a). Finally, the rats remained in the oxygenated plastic box for about 30 min after surgery until recovery from the anesthesia.

Thirty days after the surgery, the rats were anesthetized again and submitted to euthanasia as previously described (Seidel, 1994). A subcostal abdominal incision followed by the release of diaphragm muscle (Burri and Weibel, 1971) and retraction of the anterior thoracic wall were performed to facilitate the analysis of the intrathoracic viscera and the possible alterations that could occur during the postoperative period. The lung was extracted and weighed (in air and submerged in liquid) according to the technique also described by Brody and Buhain (1972). The right and left lungs of the C-NO, C-SO, D-NO and D-SO groups as well as the right lungs of C-PE and D-PE groups, were weighed twice. Immediately, the lungs were submerged in saline solution and weighed again. In order to perform volumetric evaluation, the lung weighs in the air and saline solution were taken into account (Seidel et al. 1997b). Considering that the specific weight of the isotonic saline solution was 0.987g/mL at 30°C, the following equation was applied for each piece (Scherle, 1970): V=WA-WL/GL. V= volume of the lung; WA= weight of the lung in the air (g); WL= weight of the lung in the liquid (g); GL= specific weight of the liquid (g/mL). Student "t" test and analysis of the variance completed by Tukey's test, Kruskal-Wallis and Mann-Whitney were employed. A 95% level of confidence ($p \leq 0.05$) was accepted for all comparisons. The results were referred as means ± standard deviation.

Table 1 - Glycemia (mg/dL) after six (6^{th}) and 36 (36^{th}) days of the injection of alloxan. The results were referred as means ± standard deviation (SD).

	Non-operated (NO)		Sham operation (SO)		Pneumonectomy (PE)	
	6^{th}	36^{th}	6^{th}	36^{th}	6^{th}	36^{th}
Mean	348.33	338.33	379.13	361.47	376.07	338.27
SD	44.47	59.70	66.63	62.91	74.58	52.91

Student "t" test for paired data (6^{th} day x 36^{th} day) $t_{critical} = 2.15$ no p value

Variance Analysis for Independent Groups (NO x SO x PE) $F_{critical} = 3.23$ no p value

RESULTS AND DISCUSSION

DM may be induced in rats by administration of various diabetogenic agents or by pancreatectomy. The most common diabetogenic agents used are alloxan or streptozocin (Corkill et al., 1943). *Diabetes* induced by alloxan is a well-accepted rat model to study this disease (Akimoto et al., 2000) although a periodical control of the hyperglycemia is necessary since the possibility of the normalization of glycemia must be considered (Lazarow, 1952). As shown in Table 1, a single dose of alloxan (45 mg/Kg) was effective to induce and maintain diabetes in all animals. Non diabetic rats also maintained glycemia after six (6^{th}) and 36 (36^{th}) days of the injection of saline (results not shown).

The left lateral thoracotomy and lung resection technique used in this work were similar to those described by Brody and Buhain (1973) and Nattie et al. (1974) that executed left pneumonectomy without oro-tracheal intubation. Since we did not observe complications or deaths during or after surgery, we could suggest that the decision to maintain the rats under spontaneous ventilation with rich oxygen environment for 10 min, during the surgery and the anesthesia recovery phase, gave satisfactory conditions to pneumonectomy. By using young rats subjected to lung resection (25% of total right lung volume), Berger and Burry (1985) demonstrated that the remaining lung re-expanded rapidly first by an overinflation of the airspaces and after day 4 by an increase in tissue mass and capillary volume. On days 9 and 12, the operated lungs did not differ quantitatively from control lungs. On day 18, lobectomy-lungs were smaller than controls, but on day 30, the left lung was significantly different in structure from the control lung. In contrast, Ofulue et al. (1988) using undernourished diabetic rats treated with insulin during 7 weeks reported smaller volume and weight in the remaining lung.

Table 2 - Lung weight expressed by the percentage of the differences (Δ%) under air and submerged liquid conditions calculated from [Δ % = $\frac{\text{Air} - \text{Liquid}}{\text{Liquid}}$ x 100]. The results were referred as means ± standard deviation (SD).

	Non-operated (NO)			Sham operation (SO)			Pneumonectomy (PE)		
	Air	Liquid	Δ%	Air	Liquid	Δ%	Air	Liquid	Δ%
Non-diabetic group (C group)									
Mean	1.21	1.04	16.3	1.35	1.16	16.3	1.25	1.06	17.9
SD	0.11	0.12	7.98	0.16	0.2	11.5	0.26	0.28	8.95
Diabetic group (D group)									
Mean	1.26	1.06	18.8	1.68	1.55	8.38	1.04	0.89	16.85
SD	0.16	0.18	7.33	1.58	1.59	5.97	0.18	0.18	8.61

Variance Analysis for independent groups (NO x SO x PE) $F_{critical} = 3.23$ no p value
Kruskal–Wallis Variance Analysis test. $H_{critical} = 5.99$ $H_{calculated} = 6.57^*$ (D group) SO<NO and PE
Student "t" test for C x D group) $t_{critical} = 2.05$ $t_{calc} = 2.49^*$ Pneumonectomy (C > D group)

Table 3 - Lung volumes (mL) obtained from [V = (WA – WL)/GL] for diabetic (D group) and non-diabetic group (C group). V= volume of the lung; WA= weight of the lung in the air (g); WL= weight of the lung in the liquid (g); GL= specific weight of the liquid (g/mL).

C group			**D group**		
Non-operated (NO)	Sham operation (SO)	Pneumonectomy (PE)	Non-operated (NO)	Sham operation (SO)	Pneumonectomy (PE)
0.1722	0.2195	0.1958	0.1891	0.1276	0.1382

Kruskal–Wallis's Variance Analysis test (NO x SO x PE) $H_{critical} = 5.99$ $H_{calc} = 8.14^*$ (D Group) NO > SO and PE.
Mann–Whitney test (C x D) $U_{critical} = 64$ $U_{calc} = 41.5^*$ (SO) C > D $U_{calc} = 64^*$ (PE) C > D

In agreement with these results we also found compensatory lung growth thirty days after the surgery in PE-non diabetic rats. In contrast PE-diabetic rats showed smaller lung weight (Table 2) and volume (Table 3) than PE-non-diabetic rats.
Thus, we concluded that in spite of the fact that diabetes usually was associated with elevated levels of GH and adrenal steroids, hormones which stimulated lung growth postpneumonectomy, insulin deficiency could overcome these endocrinal changes and prevents the compensatory lung growth of the remaining lung in alloxan diabetic rats.

RESUMO

Nosso objetivo foi investigar a resposta do pulmão remanescente à pneumonectomia. Para alcançar este propósito, ratas da linhagem Wistar receberam uma injeção intraperitoneal de aloxana (Grupo D) ou salina (Grupo C). Seis dias após a administração de aloxana (45 mg/kg) o *diabetes mellitus* foi confirmado e os animais aleatoriamente distribuídos em 3 subgrupos: não operados, falsamente operados e submetidos a pneumonectomia. Trinta e seis dias após a cirurgia os animais foram sacrificados e o peso e volume dos pulmões avaliados. Animais diabéticos pneumonectomizados apresentaram menor volume e peso pulmonar em relação a animais não diabéticos pneumonectomizados. Assim, os resultados demonstraram que o crescimento pulmonar compensatório que ocorre após a pneumonectomia não foi observado em animais diabéticos.

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